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THE ACTIVITY OF SELECTED SOIL ENZYMES, AND SOIL CONTAMINATION WITH ZINC, CADMIUM AND LEAD IN THE VICINITY OF THE ZINC SMELTER "MIASTECZKO SLASKIE"

AKTYWNOŚĆ WYBRANYCH ENZYMÓW GLEBOWYCH ORAZ ZANIECZYSZCZENIE GLEBY CYNKIEM, OŁOWIEM I KADMEM W SĄSIEDZTWIE HUTY CYNKU „MIASTECZKO ŚLĄSKIE"

Abstract: Heavy metals are a serious source of soil pollution, especially in the vicinity of metal smelters. This study examines the effect of selected heavy metals on the activity of soil enzymes (acid phosphatase, dehydrogenase, β-glucosidase, urease and protease) in topsoil (0–30 cm) in the vicinity of the zinc smelter "Miasteczko Slaskie". Soil enzyme activity in the polluted site was compared with a non-polluted site in the outskirts of the Pazurek nature reserve (near Olkusz). Soil samples were collected in coniferous communities. The activity of soil enzymes were compared at distances of 0.5 km, 1 km and 1.5 km from the emitter. In the vicinity of the emitter (all distances) Cd, Pb and Zn levels in topsoil exceeded acceptable limits. Also, Pb content at a non-polluted site (Pazurek nature reserve) was higher than permissible levels. Zn (HNO_3 extracted) were the highest in the topsoil samples collected at a distance of 1.5 km from the Miasteczko Slaskie plant, Cd (HNO_3 and $CaCl_2$ extracted) and Pb ($CaCl_2$ extracted) contents were highest at the distance 1 km from the emitter. However, the pollution index in the superficial soil layer (0–10 cm) was similar at all three distances from the emmiter. The Zn, Cd and Pb bioavailable concentrations ($CaCl_2$ extracted) ranged: 15–220, 0.1–7.2, 2.8–55.7 mg $\cdot$ kg^{-1} respectively. The greatest decreases were found for acid phosphatase, dehydrogenase and protease activity at the distance of 0.5 km from the emitter. Results show that soil enzymatic activity should accompany basic chemical analysis to track disorders and adverse effects in polluted soil.

Keywords: soil enzyme activity, heavy metals

Heavy metal pollution is a major environmental concern. In recent decades there has been an increasing interest in the long term effect of heavy metals at high concentration due to their persistence in soil for tens or even thousands of years [1, 2]. The presence of heavy metals in soil may influence biochemical processes by affecting both microbial

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proliferation and enzyme activity. Comparative studies have reported reductions in microbial biomass or soil enzyme activity in acidic forest soils exposed to long-term atmospheric deposition of metals from smelters [1, 3]. Microbiologically mediated processes, catalyzed by enzymes, are essential for the functioning of soil, providing the basis of carbon, nitrogen, phosphorous and sulfur cycles in soil. Therefore soil enzymes can be used as responsive indicators of the impact of metals on soil biochemical properties involved in carbon and nutrient cycles [4, 5]. In this study, we investigated the effect of soil pollution by Zn, Pb and Cd on the activities of acid phosphatase, dehydrogenase, β-glucosidase, urease and protease in topsoil of coniferous sites along a deposition gradient in the vicinity of the zinc plant "Miasteczko Slaskie". Soil enzyme activity in the polluted site was compared with a non-polluted site in the outskirts of the Pazurek nature reserve (near Olkusz). To this end, we determined and compared soil levels of Zn, Pb and C (acid extracted and potentially bioavailable).

Material and methods

Five topsoil samples from soil layers 0–10 cm, 10–20 cm and 20–30 cm were collected from the vicinity of the zinc smelter "Miasteczko Slaskie" (at distances of 0.5 km (M1), 1 km (M2) and 1.5 km (M3)). In June 2009 samples were collected from 5 random sites from an area of 50 m^2, each from a eastern direction. Soil was also collected from a coniferous forest ecosystem on the perimeter of the Pazurek nature reserve near Olkusz (P), selected as a potentially contamination-free control area. The soil enzyme activity was determined in soil samples at field moisture, sieved through a 2 mm sieve and stored at 4 ^oC before microbial analysis. The activity of alkaline and acid phosphatase was measured according to the method of Schinner et al [6]. The *p*-nitrophenol (NP) released by phosphomonoesterase activity was extracted and colored with sodium hydroxide and determined photometrically at λ = 400 nm. The phosphatase activity was expressed in μg *p*-nitrophenol (*p*-NP) g^{-1} d.m. h^{-1} (d.m. – dry matter). The urease activity estimation was based on the colorimetric determination of ammonium formation after enzymatic urea hydrolysis (10 % solution, λ – 630 nm). Urease activity was expressed as $\mu gN \cdot g^{-1}$ d.m. [6]. Protease activity was determined using casein as substrate. Aromatic amino acids react with Folin-Ciocalteu's phenol reagent in alkaline solution to form a blue complex which was determined colorimetrically at λ = 700 nm. Protease activity was expressed in μg tyrosine g^{-1} d.m. h^{-1}. Saligenin released from salicin (β-glucosido-saligenin) was determined colorimetrically after coloring with 2,6-dibromchinon-4-chlorimide, at λ = 578 nm in β-glucosidase activity estimation. β-glucosidase activity was expressed as μg saligenin g^{-1} d.m. h^{-1}. Triphenyltetrazolium chloride was the substrate which was used for the dehydrogenase activity determination. Triphenyl formazan (TPF) was extracted with acetone and measured photometrically at λ = 546 nm. The dehydrogenase activity was expressed in μg triphenyl formazan (TPF) g^{-1} d.m. h^{-1} Schinner et al [6]. The metal concentrations in the soil samples were estimated according to the method of Bouwman et al [7] and Ostrowska et al [8]. The air-dried soil samples were sieved through a 1 mm sieve and used for metal extraction with 0.01 M $CaCl_2$ (potentially bioavailable elements) or with 10 % HNO_3 (acid

extracted elements). During $CaCl_2$ extraction, 5 g of soil with 50 cm^3 of 0.01 M $CaCl_2$ solution was shaken for 5 h. An HNO_3-extractable fraction was obtained by shaking a sample (10 g) with 100 cm^3 of 10 % HNO_3 for 1 h. The contents of metals were measured in the filtered extracts. The *soil pollution index* (SPI) was calculated for each locality according to the equation given below [9] and was based on limit values as reported in [16]. The limit values (permissible concentrations) of heavy metals in soils were: Zn – 300 mg · kg^{-1}, Pb – 100 mg · kg^{-1}, Cd – 4 mg · kg^{-1}.

$$SPI = \frac{1}{n} \cdot \sum_{i=1}^{n} \cdot 100 \cdot \frac{VS_i}{LS}$$

were: n – number of elements,
VS – content of an element in the soil [mg · kg^{-1}],
LS – limit value for an element in the soil [mg · kg^{-1}].

The results are presented as means of five replicates of each estimation, together with standard deviation (SD) of the means. The data was processed using Statistica software to compute significant statistical differences between samples ($p < 0.05$) according to Tukey's multiple range test and to compute Pearson's correlation coefficients.

Results and discussion

In most cases heavy metal concentrations in the fractions of soil extracted with HNO_3 and in the fractions of soil extracted with $CaCl_2$ decreased with soil depth. Generally the highest investigated metal content were found in the superficial soil layer (0–10 cm). The highest Zn contents (acid extracted) were found 1.5 km from the emitter – the zinc smelter "Miasteczko Slaskie". The highest Pb ($CaCl_2$ extracted) and Cd contents were found at a distance of 1 km from the emitter. In acid extracted soil fractions heavy metal contents ranged for Zn 19.7–1062.3, for Cd 0.8–27.3 and for Pb 37.1–2518.5 mg · kg^{-1}, respectively. Several to almost two hundred times lower concentrations of the metals examined were measured in the potentially bioavailable fractions ($CaCl_2$ extracted) (Table 1). However the pollution index (estimated for acid and $CaCl_2$ extracted metal concentrations) in the superficial soil layer (0–10 cm) was at a comparable level at all distances from the emitter (except M2, HNO_3). HNO_3-extraced heavy metals in the vicinity of the emitter (at all distances 0–10 cm) were higher than acceptable limits for soil according to the Environmental Regulation (2002) (Zn 300 mg · kg^{-1}, Pb 100 mg · kg^{-1} and Cd 4 mg · kg^{-1}). In our study Pb content in the samples collected in the similar coniferous forest ecosystem on the perimeter of the Pazurek nature reserve in the soil layer 0–20 cm was above the permissible concentration [10]. In our previous study in the vicinity of the former "Szopienice" plant in Katowice, we found higher Zn and Cd concentration and lower Pb concentration [4]. In the superficial soil layer in the vicinity of the Szopienice plant and at a calamine site in Dabrowa Gornicza and in zinc wastes heap located in Katowice, the bioavailability of the metals was low and at comparable levels to this present study. Olszowska et al found similar levels of heavy metals in the soil of the pine coniferous forest of age class III in threat

Table 1

Mean heavy metal content in the surface soil layer in the vicinity of zinc smelter Miasteczko Slaskie and in Pazurek. Values with the same letter are statistically the same for $p < 0.05$

Sampling sites	M1 (0–10)	M1 (10–20)	M1 (20–30)	M2 (0–10)	M2 (10–20)	M2 (20–30)	M3 (0–10)	M3 (10–20)	M3 (20–30)	P (0–10)	P (10–20)	P (20–30)
Zn (HNO_3)	419.3 ± 26.9 j	218.6 ± 6.7 d	151.0 ± 1.3 c	919.9 ± 60.4 i	765.6 ± 4.1 h	233.2 ± 11.1 d	1062.3 ± 66.7 g	520.3 ± 51.4 f	188.4 ± 6.4 e	127.9 ± 16.4 c	54.8 ± 2.6 b	19.7 ± 0.2 a
Zn ($CaCl_2$)	198.7 ± 4.3 g	89.5 ± 1.6 d	98.7 ± 2.8 e	97.9 ± 2.5 e	220.1 ± 6.2 f	101.7 ± 1.2 e	140.4 ± 24.9f	124.9 ± 9.6 f	85.1 ± 2.2 d	46.42 ± 1.34 c	21.8 ± 0.3 b	15.1 ± 1.7 a
Cd(HNO_3)	7.9± 0.3d	2.6 ± 0.8 ab	4.9 ± 0.3 c	27.3 ± 0.6 g	16.4 ± 1.3 f	8.8 ± 0.4 d	19.5 ± 0.4 h	11.41 ± 0.19 e	3.9 ± 0.1bc	1.8 ± 0.1a	3.7 ± 0.4 bc	0.8 ± 0.01a
Cd ($CaCl_2$)	4.8 ± 0.7 c	2.3 ± 0.3 b	3.4 ± 0.1 c	7.2 ± 4.1 d	4.7 ± 0.1 c	3.2 ± 0.1 c	4.4 ± 0.5 c	2.1 ± 0.1 b	1.1 ± 0.1 b	1.6 ± 0.1 b	0.34 ± 0.2 a	0.13 ± 0.01 a
Pb (HNO_3)	2055.5 ± 151.5 i	355.7 ± 1.2 d	2518.5 ± 134.5 h	569.8 ± 7.1 g	431.9 ± 6.5 f	291.1 ± 22.4 c	1809.1 ± 43.2 j	388.1 ± 0.8 e	247.7 ± 12.5bc	229.1 ± 3.7 b	195.5 ± 13.1 b	37.1 ± 0.05a
Pb ($CaCl_2$)	16.5 ± 3.1 b	16.8 ± 0.6 b	13.2 ± 2.3 b	55.7 ± 19.2 a	41.8 ± 0.5 a	7.4 ± 0.1 c	16.2 ± 1.7 b	3.6 ± 0.1 c	6.5 ± 0.3 c	5.9 ± 1.1c	2.8 ± 0.2 c	4.0 ± 0.4 c
Pollution index (HNO_3)	797.9 ± 50.9 e	164.6 ± 6.2 f	897.8 ± 47.2 e	519.4 ± 4.0 j	365.3 ± 9.5 h	197.0 ± 12.4 g	883.2 ± 25.3 e	282.2 ± 7.5 d	136.5 ± 5.3c	105.8 ± 0.5 b	102.0 ± 7.2 b	21.2 ± 0.04 a
Pollution index ($CaCl_2$)	67.8 ± 7.1 e	35.0 ± 2.2 c	43.7 ± 0.3 d	89.4 ± 40.4 ef	77.3 ± 1.6 f	40.4 ± 0.6 d	57.5 ± 7.3 e	32.0 ± 1.6 c	20.5 ± 0.8 b	20.1 ± 0.5 b	6.2 ± 1.6 a	4.1 ± 0.04 a

Table 2

Mean pH value. organic matter content in the surface soil layer the vicinity of zinc smelter Miasteczko Slaskie and in Pazurek. Values with the same letter are statistically the same for $p < 0.05$

Sampling sites	M1 (0–10)	M1 (10–20)	M1 (20–30)	M2 (0–10)	M2 (10–20)	M2 (20–30)	M3 (0–10)	M3 (10–20)	M3 (20–30)	P (0–10)	P (10–20)	P (20–30)
pH	5.63 ± 0.01a	5.66 ± 0.04 a	5.63 ± 0.02 a	5.25 ± 0.01 b	5.31 ± 0.01 c	5.425 ± 0.005 d	5.82 ± 0.01 e	5.985 ± 0.04 f	5.805 ± 0.01 g	5.21 ± 0.01 h	5.555 ± 0.01 i	5.42 ± 0.01j
Organic matter	3.3 ± 0.02 a	1.25 ± 0.09 b	1.32 ± 0.08 b	15.35 ± 0.03c	10.76 ± 0.02 d	3.34 ± 0.01 a	5.955 ± 0.06 e	4.39 ± 0.03 f	2.795 ± 0.01 g	7.055 ± 0.03 h	5.52 ± 0.18 i	1.17 ± 0.02 j

Table 3

Mean soil enzyme activity in the surface soil layer in the vicinity of zinc smelter Miasteczko Slaskie and in Pazurek. Values with the same letter are statistically the same for $p < 0.05$

Sampling sites	M1 (0–10)	M1 (10–20)	M1 (20–30)	M2 (0–10)	M2 (10–20)	M2 (20–30)	M3 (0–10)	M3 (10–20)	M3 (20–30)	P (0–10)	P (10–20)	P (20–30)
Acid phosphatase [μg *p*-NP $g^{-1} \cdot h^{-1}$]	3.0 ± 0.1 a	4.4 ± 0.4 a	20.7 ± 2.4 b	208.2 ± 24.9 c	42.5 ± 0.0 d	72.4 ± 2.0 e	31.0 ± 7.5 bg	25.9 ± 2.5 b	25.5 ± 2.4 b	109.3 ± 24.1 f	37.3 ± 0.8 g	8.5 ± 2.4 h
Dehydrogenase [μg TPF g^{-1} d.m. h^{-1}]	1.3 ± 0.0 a	2.5 ± 1.0 a	4.8 ± 1.0 bc	10.0 ± 1.1 e	3.7 ± 0.4 b	7.3 ± 0.9 d	12.6 ± 1.8 e	20.3 ± 1.8 f	4.1 ± 0.6 b	19.8 ± 1.5 f	12.6 ± 1.0 e	6.6 ± 0.2 cd
Urease [μgN g^{-1} d.m. h^{-1}]	80.1 ± 3.7c	39.2 ± 5.1a	82.2 ± 0.9 c	181.4 ± 15.7 e	137.1 ± 7.6 d	94.8 ± 8.1c	135.9 ± 12.3 d	71.6 ± 17.2 bc	42.7 ± 1.2 a	67.4 ± 0.9 b	44.0 ± 4.8 a	71.1 ± 10.9 bc
β-Glucosidase [μg saligenin g^{-1} d.m. h^{-1}]	5573.3 ± 293.3 g	1361.7 ± 179.1 ab	1741.4 ± 3.1 c	1023.0 ± 158.0 a	1447.8 ± 11.4 b	2005.4 ± 27.1 e	4266.5 ± 347.5 f	1717.0 ± 88.0 c	4060.6 ± 805.8 f	1913.0 ± 14.4 d	2292.9 ± 227.1 e	1176.0 ± 130.7 a
Protease [μg tyrosine g^{-1} d.m. h^{-1}]	2.4 ± 0.4 de	0.3 ± 0.1 a	0.7 ± 0.0b	4.3 ± 0.6 ef	3.1 ± 0.6e	1.9 ± 0.4 cd	2.1 ± 0.0 d	6.0 ± 0.6 g	1.4 ± 0.2 c	4.8 ± 0.6 f	5.4 ± 0.5 f g	0.5 ± 0.2 a

Table 4

Correlation coefficients between heavy metal content (acid extracted and bioavailable) in studied soil samples and enzyme activity. (Significant for $p < 0.05$)

	Zn (HNO_3)			Zn ($CaCl_2$)			Pb (HNO_3)			Pb ($CaCl_2$)			Cd (HNO_3)			Cd ($CaCl_2$)		
Soil layer	0–10	10–20	20–30	0–10	10–20	20–30	0–10	10–20	20–30	0–10	10–20	20–30	0–10	10–20	20–30	0–10	10–20	20–30
Acid phosphatase			0.79	–0.64		0.62	–0.8			0.72			0.48	0.6	0.93			0.6
Dehydrogenase				–0.87			–0.73				–0.71	–0.48				–0.48	–0.51	
Urease	0.84	0.88			0.91			0.66		0.75	0.8		0.9	0.92	0.54	0.54	0.85	0.63
β-Glucosidase		–0.54	0.5	0.86	–0.66		0.92	–0.83		–0.48	–0.61						–0.74	
Protease	–0.41		0.84	–0.81		0.6	–0.92				–0.48				0.78			

zone III in the region of the activity of the smelter "Miasteczko Slaskie" (Zn – 437 mg · kg^{-1} d.m., Pb – 1002 mg · kg^{-1} d.m., Cd – 3.90 mg · kg^{-1} d.m.) [11].

The concentrations of Zn, Pb and Cd extracted with 0.1 M $NaNO_3$ (considered as bioavailable) in the study of the upper layer of the 100-year-old zinc-lead waste heap were also low and accounted for no more than 0.5 % of total concentrations in the heap material [12].

Determination of soil enzymatic activity can serve as a basis for evaluation of soil quality as enzymes are particularly susceptible to changes in the environment [13]. In this present study the greatest decreases were found for acid phosphatase, dehydrogenase and protease activity at a distance of 0.5 km from the zinc smelter "Miasteczko Slaskie". The activity of β-glucosidase was lowest at a distance of 1 km from the emitter. Urease activity in soil collected at the distance of 0.5 km from the emitter was at a comparable level with the urease activity in the soil of non-polluted site. Many reports demonstrated significant inhibition of enzymatic activities by heavy metals. However, the effects of heavy metals on enzyme activity may vary considerably among the elements, enzymes and soils [1, 14]. The negative correlations between particular extracted heavy metal content and enzyme activity were found only in some cases (especially between Pb content (HNO_3 extraction), Zn content ($CaCl_2$ extraction) pollution index (HNO_3) and acid phosphatase, dehydrogenase and protease activity in the superficial layer (0–10 cm) (Tables 4, 5).

Table 5

Correlation coefficients between pollution index (established for acid extracted and bioavailable metals content) in studied soil samples and enzyme activity. (Significant for $p < 0.05$)

	Pollution index (HNO_3)			Pollution index ($CaCl_2$)		
Soil layer	0–10	10–20	20–30	0–10	10–20	20–30
Acid phosphatase	–0.54					0.6
Dehydrogenase	–0.7			–0.59	–0.5	
Urease		0.89		0.56	0.88	0.53
β-Glucosidase	0.71	–0.56			–0.71	
Protease	–0.87					

In many cases positive correlation coefficients between organic matter content and examined soil enzyme activity were stated (Table 6).

Some studies demonstrated a significant relationship between soil enzymes and other soil characteristics, but the relationship is largely dependent on the species of enzyme and environmental variables [15]. The content of organic substance or the soil pH can have a significant influence on the decrease of available forms of heavy metals and their biological inactivation [16, 17]. There are reports indicating that enzymes involved in the C cycle are less affected by heavy metals in contaminated soils than enzymes related to N, P, and S cycles and the expected reduction of activity is often not observed [1]. β-glucosidase activity was less affected in our study. However, intracellular activity of dehydrogenase involved in the C-cycle, was decreased in the emitter vicinity.

Extracellular enzymes adsorbed onto clay minerals can act for a long time in the soil because of increasing durability and denaturation and proteolysis resistance [18].

Table 6

Correlation coefficients between pH and organic matter content in studied soil samples and enzyme activity. (Significant for $p < 0.05$)

	pH			Organic matter content		
Soil layer	0–10	10–20	20–30	0–10	10–20	20–30
Acid phosphatase	–0.79	–0.49		0.94	0.89	0.83
Dehydrogenase	–0.47	0.75	–0.83			
Urease		–0.52	–0.72	0.78	0.88	
β-Glucosidase	0.83		0.8	–0.84		0.95
Protease	–0.93			0.58		0.92

Some authors proposed reduced enzyme activity (especially dehydrogenase, urease and phosphatase) as indicators of soil contamination with heavy metals [1]. This statement has been confirmed by our study in the case of acid phosphatase and dehydrogenase activity.

Conclusions

The dynamics and differences in the compared soils can be tracked by measuring enzymatic activity. The low bioavailability of the studied metals may be one of the reasons for their lower effect on the enzymatic activity. The greatest negative impact of examined metals was found for dehydrogenase and acid phosphatase activity. These enzymes activity were better in estimation of negative changes in soil. Hence, the study of enzymatic activity should be conducted in forest ecosystems and especially in those under anthropogenic pressure.

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Abstrakt: Zanieczyszczenie gleby metalami ciężkim zwłaszcza w pobliżu hut metali to poważny problem. Oceniano wpływ metali ciężkich na aktywność enzymatyczną gleby. Celem badań była ocena aktywności enzymów glebowych: fosfatazy kwaśnej, dehydrogenaz, β-glukozydazy, ureazy i proteazy w wierzchniej warstwie gleby w najbliższym sąsiedztwie huty cynku Miasteczko Śląskie. Aktywność enzymatyczną gleby na stanowiskach zanieczyszczonych porównywano z aktywnością na stanowisku niezanieczyszczonym, próbki gleby pobierano również w pobliżu rezerwatu przyrody Pazurek koło Olkusza ze zbiorowisk borowych. Aktywność enzymatyczna w glebie najbliższego sąsiedztwa emitora była porównywana w odległości 0,5, 1, 1,5 km od emitora. Wykazane zawartości Cd, Pb i Zn przekraczały dopuszczalne normy przyjęte dla gleby. Zawartość Pb w próbkach gleby pobieranych w pobliżu rezerwatu Pazurek była wyższa niż dopuszczalne wartości dla gleby. Zawartość Zn (ekstrakcja HNO_3) była najwyższa w odległości 1,5 km od emitora (0–10 cm), z kolei zawartości Cd (ekstrakcja HNO_3 i $CaCl_2$) i Pb (ekstrakcja $CaCl_2$) były najwyższe 1 km od emitora. Wskaźnik zanieczyszczenia wykazany dla wierzchniej warstwy gleby (0–10 cm) był na porównywalnym poziomie w glebie w sąsiedztwie emitora. Koncentracje biodostępnych form metali (ekstrakcja $CaCl_2$) mieściły się w przedziałach odpowiednio: 15–220, 0,1–7,2, 2,8–55,7 mg $\cdot$ kg^{-1}. Najniższe aktywności fosfatazy, dehydrogenazy i proteazy wykazano w próbkach gleby pobieranej w odległości 0.5 km. Badania aktywności enzymatycznej gleby obok analiz chemicznych powinny być prowadzone w celu śledzenia zmian w glebach zanieczyszczonych.

Słowa kluczowe: aktywność enzymatyczna gleby, metale ciężkie